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1: J Chromatogr 1989 Nov 10;496(1):71-82 Related Articles, NEW Bool

Effect of stationary and mobile phases on hydrophobic interaction chromatography of proteins and peptides.

Smith JA, O'Hare M.

Department of Biochemistry, University of Liverpool, U.K.

A number of different stationary phases designed for hydrophobic interactior chromatography have been examined to assess their efficiency and resolving capability with respect to protein and peptide mixtures. A packing with an ether-bonded phase was substantially less hydrophobic than those with propy or phenyl-bonded surface chemistry. While the overall efficiencies of most columns were broadly similar with respect to most proteins, some proteins di chromatograph with enhanced efficiency on specific packings. The elution order of individual proteins was, with one or two exceptions, similar for all columns tested using comparable mobile phases. It differed, however, substantially from orders obtained with conventional reversed-phase alkyl-bonded phases and from the elution orders obtained when the hydrophobic packings were used in a reversed-phase mode, i.e. with an organic modifier gradient. Varying the salt used in the mobile phase and its pH under hydrophobic interaction conditions (high ionic strength) changed overall retentivities and also altered specific retention orders, thus offering possibilities of selective resolution of some mixtures.

PMID: 2592518 [PubMed - indexed for MEDLINE]

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1: J Chromatogr 1984 Dec 28;317:141-55

Comparison of hydrophobic-interaction and reversed-phase chromatography of proteins.

Fausnaugh JL, Kennedy LA, Regnier FE.

The variable hydrophobic nature of proteins allows their separation through differential hydrophobic surface interactions. From these observations two modes of protein chromatography have been developed, hydrophobic-interaction chromatography (HIC) and reversed-phase chromatography (RPC Selectivity of the HIC column can be easily manipulated by changing mobile phase variables. Protein retention was increased by decreasing the pH from neutrality or by using a salt with a greater "salting-out" ability. In addition, selectivity can be altered through chemical modification of the matrix surfac Protein retention and resolution decreased concomitantly with matrix ligand density. There were several major differences in HIC and RPC selectivity. Hydrophilic proteins such as cytochrome c and myoglobin were weakly retained on the HIC column but strongly retained on the RPC column. In contrast, a hydrophobic protein such as beta-glucosidase was strongly retaine on the HIC column and only weakly retained on the RPC column. Other proteins were retained equally by RPC and HIC columns. Load capacity on t HIC column was determined by plotting resolution as a function of protein load. Resolution decreased significantly after 7.5 mg of total protein had bee loaded onto the column per cm3 of column material. Samples of lactic dehydrogenase and alpha-chymotrypsin ranging in size from 10-200 micrograms were recovered from an HIC column with greater than 86% enzymatic activity in all cases. The recovery of enzymatic activity of alpha-chymotrypsin ranged from 55-91%, while none of the activity of beta-glucosidase was recovered from the RPC column.

PMID: 6530430 [PubMed - indexed for MEDLINE]

1: J Chromatogr 1988 Jul 1;444:269-74

Hydrophobic interaction chromatography of peptides as an alternative to reversed-phase chromatography.

Alpert AJ.

Poly LC, Columbia, MD 21045.

Hydrophobic interaction chromatography (HIC) was examined as an alternative to reversed-phase chromatography (RPC) for peptide separations by high-performance liquid chromatography. With small peptides, selectivity was similar in both modes. This was the case with commercially available standards and with a set of synthetic peptides having the same amino acid composition but different sequences. Column efficiency was higher in RPC. HIC possesses several other disadvantages, including significant baseline changes during gradient elution and a requirement for non-volatile mobile phases, which complicates peptide isolation. Thus, RPC is still the method of choice for most small peptides. Marked differences in selectivity were noted with small proteins and polypeptides large enough to possess tertiary structu Good results were also obtained by HIC in the case of some peptides that could not be purified at all by RPC, due to aggregation or poor binding or recovery. Thus, in these cases, HIC is a useful alternative to RPC for peptide purification.

PMID: 3204135 [PubMed - indexed for MEDLINE]

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1: J Chromatogr 1990 Feb 2;500:503-18

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Microenvironmental contributions to the chromatographic behavior of subtilisin in hydrophobic-interaction and reversed-phase chromatography.

Chicz RM, Regnier FE.

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Genetically engineered variants were used to examine how microenvironmental changes in the S1 substrate binding subsite of subtilisin contribute to chromatographic behavior of proteins on hydrophobic-interacti chromatography (HIC) and reversed-phase chromatography (RPC) columns. Gradient elution studies over a wide pH range showed that conditions could l found where a HIC support could separate proteins varying by one amino aci Although all single-site variants could not be separated by HIC, this chromatographic mode was found to be complementary to cation-exchange chromatography for the separation of such variants. RPC was found to be of much less utility in the resolution of variant proteins. Retention and resolutio of subtilisin variants was found to vary on RPC with the concentration and type of mobile phase pairing agent.

PMID: 2184168 [PubMed - indexed for MEDLINE]

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